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Dielectric properties (viz real and imaginary components of the complex dielectric constant, and the absorption coefficient) were determined for mammalian cells and viruses over the frequency range 50- to 26,400 MHz. Dielectric properties were determined by using an open probe technique which measured the complex reflection coefficient using a Hewlett Packard 8510 network analyzer and associated data processing system. Independent variables investigated included: temperature, cell cycle, metabolic inhibitors, anesthetic agents, ionophores, and fixatives. Under certain conditions, these variables affected the dielectric properties of cells or viruses. No firm evidence of resonant-like absorption of radiofrequency radiation in cell or viral macromolecules, such as DNA, has been obtained.


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ANNUAL REPORT

# RADIOFREQUENCY/MICROWAVE CELL ABSORPTION AND ACTION SPECTROSCOPY

Contract Number: N00014-84-K-0539

Report Period: September 1, 1987-September 30, 1988

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## OVERVIEW OF RESEARCH ACCOMPLISHMENTS

During the first year of study supported by this contract the principal research accomplishments are summarized as follows:

1) procedures were developed for the measurement of cell and viral dielectric properties ($\epsilon', \epsilon'', \alpha$) under controlled conditions in the frequency range 50MHz-26.4 GHz;

2) a comparison was made of measured dielectric parameters of human erthrocytes (whole blood and washed cells) and erythrocyte ghosts with: a) theoretical values predicted by Debye dielectric dispersion theory, and b) published values;

3) the temperature dependence of $\epsilon', \epsilon''$ for human erythrocytes was determined in the range 4.5-37$^{o}$C;

4) the effect of sodium azide and sodium fluoride on erythrocyte dielectric parameters was determined;

5) dielectric spectra (50MHz-26.GH4z) were determined for mammalian cell lines including: a) lymphocytes, b) HeLa, c) glioma (LN-71), d) L929, e) MKTD, f) RT-2, g) YAC, h) VERO and i) CHO;

6) dielectric spectra were measured for: a) coxsackie virus (CB4), b) herpes simplex virus (HSVI), and c) adenovirus;

7) effects of temperature, metabolic inhibitors, anesthetic agents, fixatives, and an ionophore (A23187), on cell dielectric properties were investigated;

8) to facilitate interpretation of cell dielectric data and to provide a means of applying such data to an assessment of cell electromagnetic absorption and action spectra, a quasi-steady state solution of Laplace's equation was obtained to provide a means of calculating induced electrical potentials and dielectric constants in multicompartment models of mammalian cells. A computer program was written to permit calculation of induced cellular potentials resulting from incident harmonic (sinusoidal) radiofrequency electromagnetic fields;

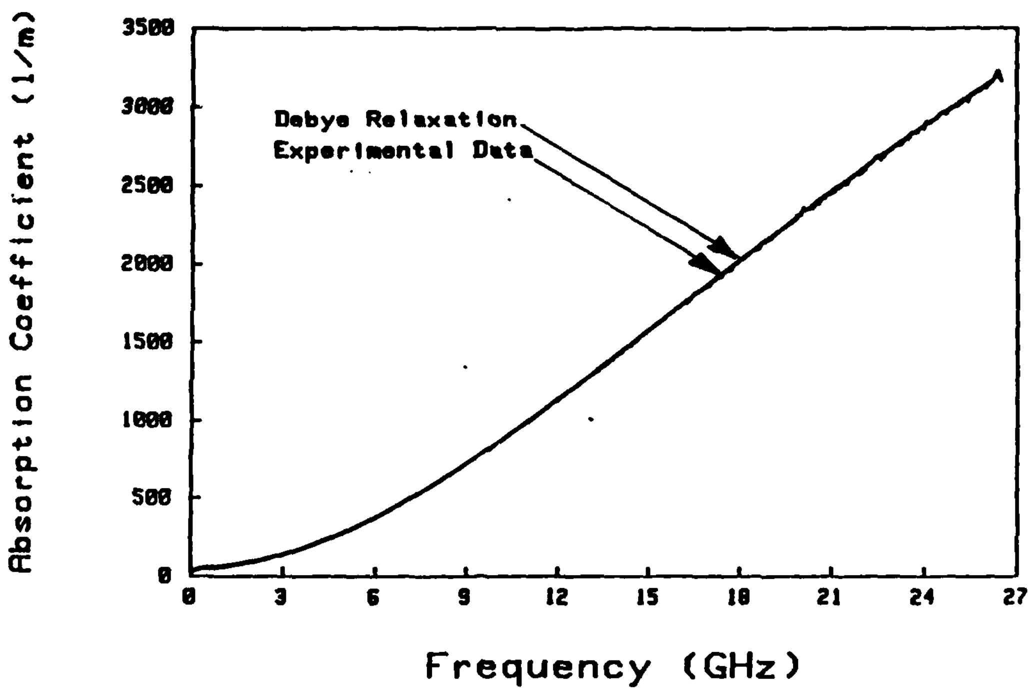
9. cell and viral dielectric spectra were analyzed to identify frequency regions in which there was evidence of: a) DNA resonant absorption, b) enhanced absorption due to DNA or other biomacromolecules, c) anomalous dispersions (i.e. dispersions not predicted by theory). Attention was focussed on the 1- 10GHz frequency range due to previously reported DNA resonant absorption in this region;

10. procedures were developed and applied to the synchronization of Chinese hamster ovary (CHO) cells. The dielectric properties of synchronized cells in various stages of the cell cycle were determined and compared with those of unsynchronized CHO cells.

## SPECIFIC OBJECTIVES

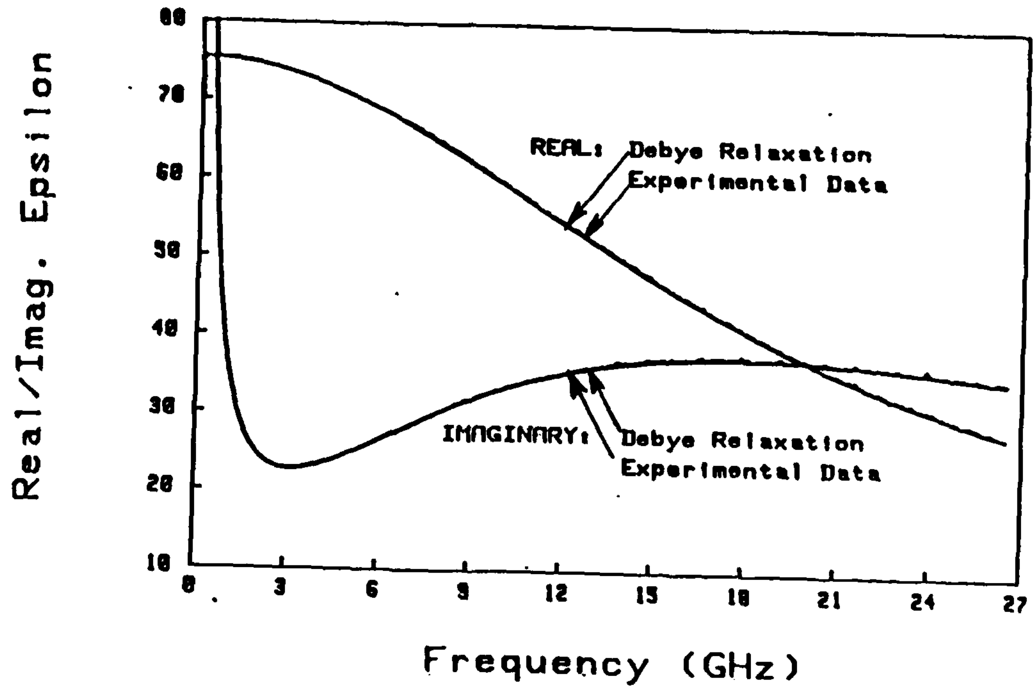
1.) Measurement Procedures

Procedures were developed to permit precise and accurate measurement of cell and virus dielectric parameters using a Hewlett Packard 8510 network analyzer, coaxial probe, and associated digital computer storage, analysis, and display systems. Optimization involved investigating the effects of variation of: a) cell sample volume, b) cell concentration, c) dielectric probe size, type, and position in sample, d) stirring during measurement, and e) sample temperature control. Successful completion of this phase of the investigation has enabled us to obtain measurements of cell dielectric parameters ($\epsilon'$,$\epsilon''$,$\alpha$) of human erythrocytes for comparison with published values, thus permitting a determination of the accuracy of the measurement procedures. In addition, measurement and calibration accuracy were evaluated by comparing the dielectric parameters ($\epsilon'$,$\epsilon''$,$\alpha$) of a 0.154M saline solution with values predicted theoretically by Debye dispersion theory. Comparisons of measured versus theoretical dielectric parameters were made over a frequency range of 50MHz to 26.4 GHz at temperatures of from 4.5 to 38$^{o}$C. Graphical comparison of measured and theoretical values of $\epsilon'$,$\epsilon''$ and $\alpha$ (absorption coefficient) for a 0.154 M NaCl solution at 22.5$^{o}$C shown in Figures 1 and 2, indicate the high degree of accuracy of this determination.




Comparison of Debye Dispersion vs. Experimental Values of Absorption Coefficient for 0.15M NaCl, 22.5°C.

Figure 1




Comparison of Debye Dispersion vs. Experimental Values of Real/Imaginary Epsilons for 0.2M NaCl, 22.5°C Using Frequency Domain Liquid Calibration Technique.

Figure 2

Determination of the effect of temperature variation on cell dielectric spectra over this frequency range indicated the need for temperature control precision of at least $\pm$ 0.1°C. Temperature control procedures providing this degree precision were developed for cell and virus measurements.

2.) Comparison of Erythrocyte Dielectric Parameters with Published Values

The accuracy of the measurement system was evaluated by comparing measured values with values reported by Stuchly and Stuchly (1980) and Cook (1952). Table 1 indicates the degree of agreement of our data with published values. Verification of our sampling and measurement procedures also involved determination of the dependence of $\epsilon'$,$\epsilon''$ and $\alpha$ on erythrocyte concentration. Dielectric spectra were determined for whole blood and washed erythrocytes suspended in isotonic saline at concentrations of $5x10^9$-, $5x10^8$-, $5x10^6$, $5x10^5$ cells/ml. Dielectric dispersion mixture equations were used to compare measured with theoretically predicted values. Cell dilution studies with erythrocytes, and other cell types, were used to determine the sensitivity of the dielectric dispersion measurement system. It was determined that the practical concentration limit was $10^6$ cells/ml; the minimum concentration that may be meaningfully resolved from the cell suspension medium is approximately $5x10^5$ cells/ml.

TABLE I. Comparison of the Dielectric Constants of Human Blood Determined in this Study with Published Values*

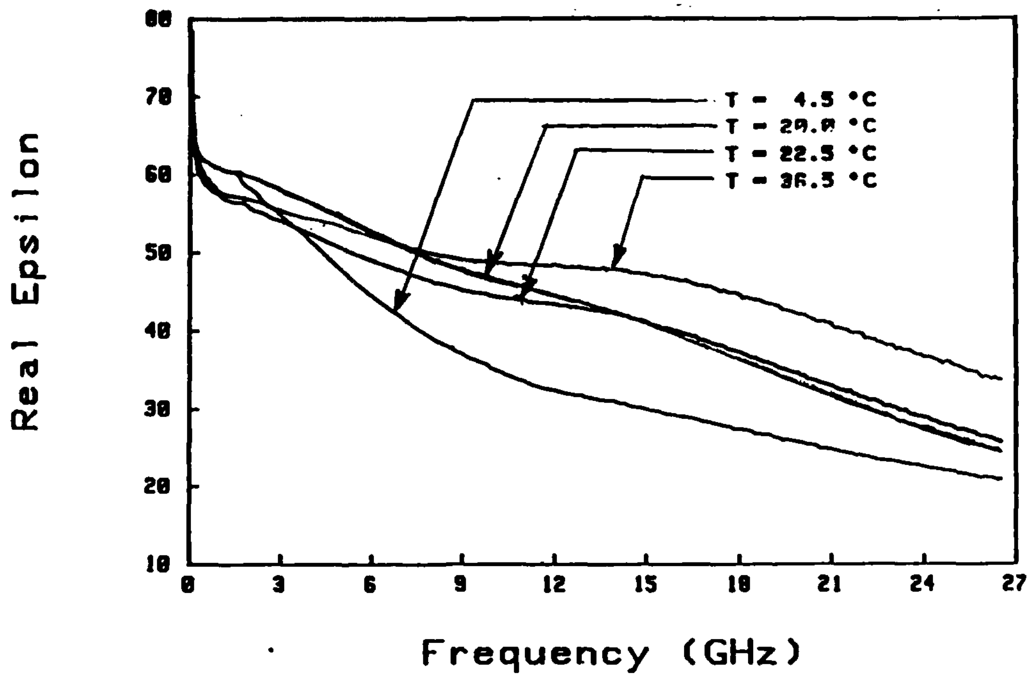
| Frequency (MHz) | Measured Value and (Temperature (°C)) | Literature Value and (Temperature (°C) ) |
|---|---|---|
| **A. Real Part of Complex Dielectric Constant ($\epsilon'$)** | | |
| 100 | 78.0 (36.5 | 72.0 (37) |
| | 75.2 (20) | 75.0 (23) |
| 3000 | 55.5 (36.5) | 56.0 (35) |
| | 54.0 (22.5) | 57.5 (25) |
| 9400 | 47.0 (36.5) | 47.8 (35) |
| | 45.0 (22.5) | 45.5 (25) |
| **B. Imaginary Part of Complex Dielectric Constant ($\epsilon''$)** | | |
| 200 | 100.0 (36.5 | 108.0 (37) |
| 400 | 56.2 (36.5) | 58.5 (37) |
| 3000 | 15.4 (36.5) | 15.9 (35) |
| | 14.2 (20) | 17.1 (25) |
| 9400 | 21.3 (36.5) | 19.9 (35) |

*Stuchly, M.A. and Stuchly, S.S.: Dielectric Properties of Biological Substances - Tabulated. J. Microwave Power 15:19-26 (1980).

Cook, H.F.: A Comparison of the Dielectric Behavior of Pure Water and Human Blood at Microwave Frequencies. Brit. J. Appl. Phys. 3:249-255 (1952)

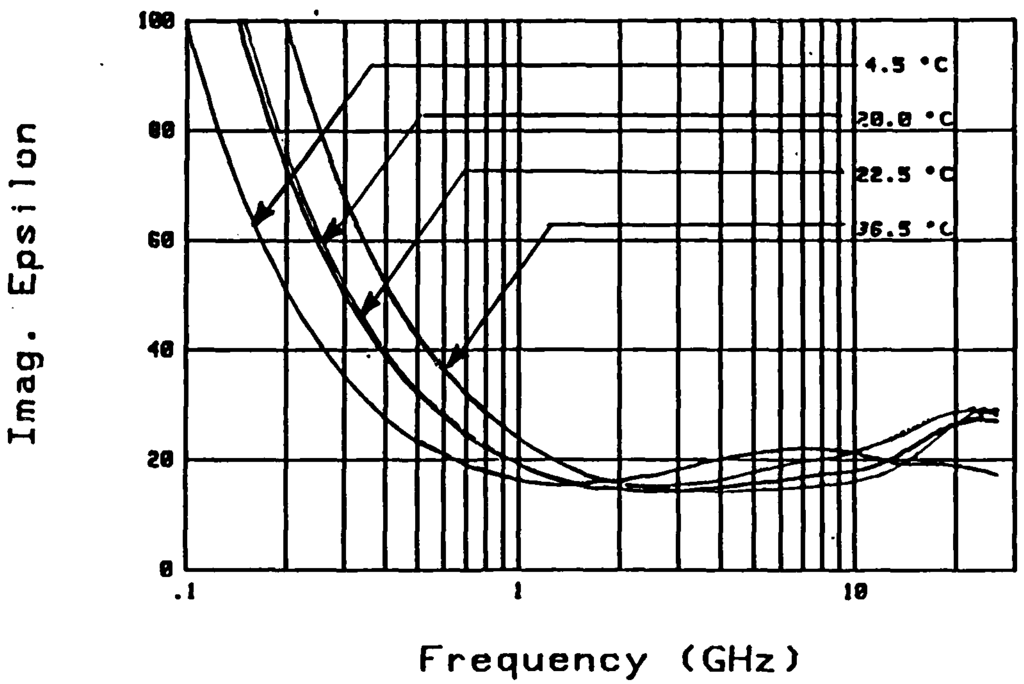
3.) Temperature Dependence of Erythrocyte Dielectric Parameters

Dielectric spectra of whole human blood (50MHz - 26.4 GHz were measured at 4.5-, 20- 22.5- and 36.5°C. Figure 3 is a graph of , the real part of the dielectric constant at these temperatures. Comparison of these data with predictions of theoretical (Debye) temperature dependence of $\epsilon'$ indicates general agreement, including an approximate temperature coefficient of 0.5% decrease in $\epsilon'/^{o}C$ at frequencies less than 5 GHz and a corresponding positive temperature coefficient at higher frequencies which results in a temperature-dependent cross over in the 3-8GHz frequency range. The experimental data, however, exhibits apparently anomalous behavior at 20- and 22.5°C, characterized by larger than predicted relative values of $\epsilon'$. Comparison of the difference in $\epsilon'$ at 20- and 22.5°C reveals a significantly larger difference and higher frequency crossover than theoretically expected. The anomalous dielectric properties of human blood at temperatures of 20- and 22.5°C was also indicated by the imaginary component of the complex dielectric constant $\epsilon''$, as shown in Figure 4. Here, as in the case of $\epsilon'$, there was an unexpectedly large negative temperature coefficient at frequencies of greater than 3GHz. Data at 4.5 and 36.5°C, on the other hand, exhibit expected frequency dependence with cross overs occurring at 2 and 15 GHz. The unexpected temperature dependence of the dielectric properties of blood was further illustrated by the absorption coefficient $\alpha$, which is shown in Figure 5 as a function of frequency for temperatures of 4.5-, 20-, 22.5-, 36.5°C. The temperature dependence of $\alpha$ is theoretically predictable since it is a function of $\epsilon'$ and $\epsilon''$, given



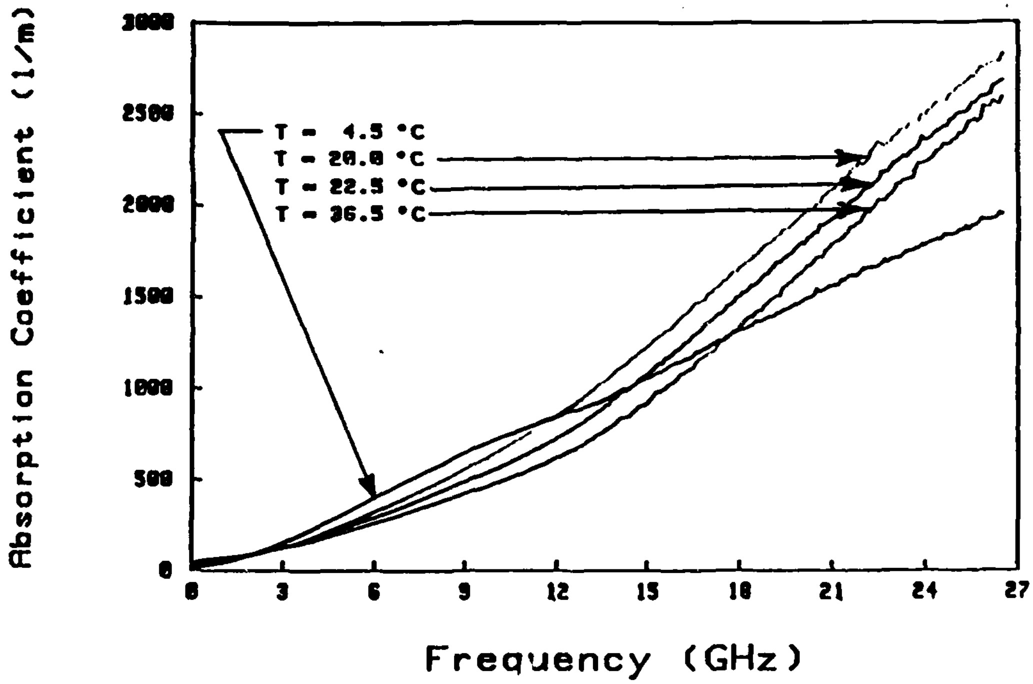

Variation of the Dielectric Parameter e' with Frequency
for Human Whole Blood at T = 4.5, 20.0, 22.5, and 36.5 °C.

Figure 3



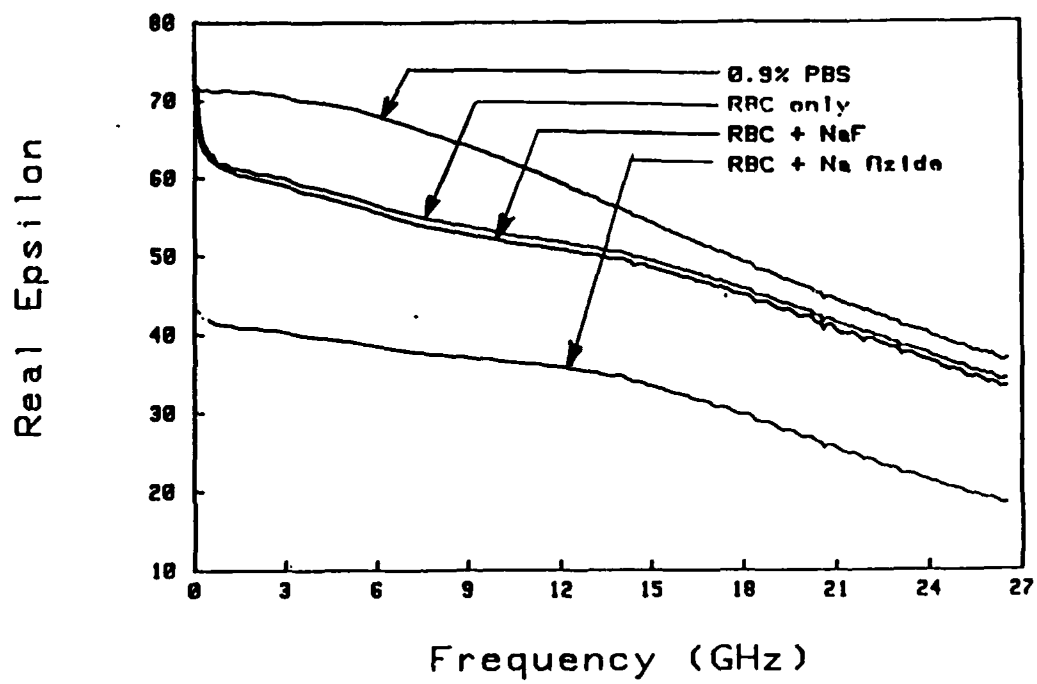

Variation of the Dielectric Parameter e'' with Frequency
for Human Whole Blood at T = 4.5, 20.0, 22.5, and 36.5 °C.

Figure 4




Variation of the Absorption Coefficient with Frequency for Human Whole Blood at T = 4.5, 20.0, 22.5, and 36.5 °C.

Figure 5




Permittivity (ε′) of RBC, RBC + NaF, and RBC + NaAzide which are suspended in 0.9% PBS buffer. T = 36.5 °C.

Figure 6

by the relationship:

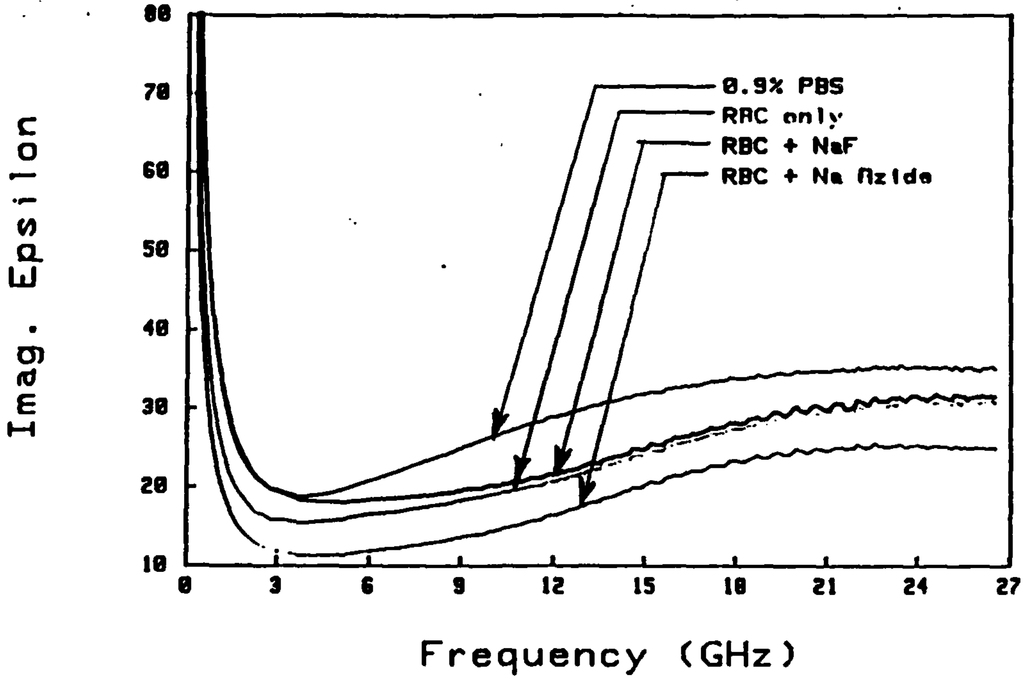
$$\alpha = \frac{2\pi f}{C_o} \left[2\epsilon' \left(1 + \left(\frac{\epsilon''}{\epsilon'}\right)^2\right)^{\frac{1}{2}} - 2\epsilon'\right]^{\frac{1}{2}}$$

The temperature and frequency dependence of the dielectric parameters of blood at 20- and 22.5°C are not predicted by Debye dispersion theory. Since we have not found published dielectric spectra for human whole blood at these temperatures and frequencies, comparisons are not possible. We have determined $\epsilon'$,$\epsilon''$ and $\alpha$ for HeLa cells ($9 \times 10^6$ cells/ml) at temperatures of 5.2-, 22.5-, and 36.5°C. Comparison of these data with predicted temperature dependence of $\epsilon$,$\epsilon''$, and $\alpha$ revealed no anomalies. An investigation of temperature-dependence of the complex dielectric constant of washed human erythrocytes, resuspended in isotonic phosphate buffered saline (PBS), also revealed anomalous behavior of $\epsilon'$ in the 20-22.5°C temperature range.

We will replicate the dielectric spectra of human blood at these frequencies and temperatures and at additional temperatures in the range 20- to 25°C. There is no explanation for these data. It should be noted that erythrocyte membrane phase transitions occur in this temperature range (ie 18-25°C) suggesting that this phenomenon may affect $\epsilon'$. However, there is no obvious mechanism for the alteration of $\epsilon'$ by a temperature-dependent membrane phase change in this frequency range, suggesting that other cell components may be involved.

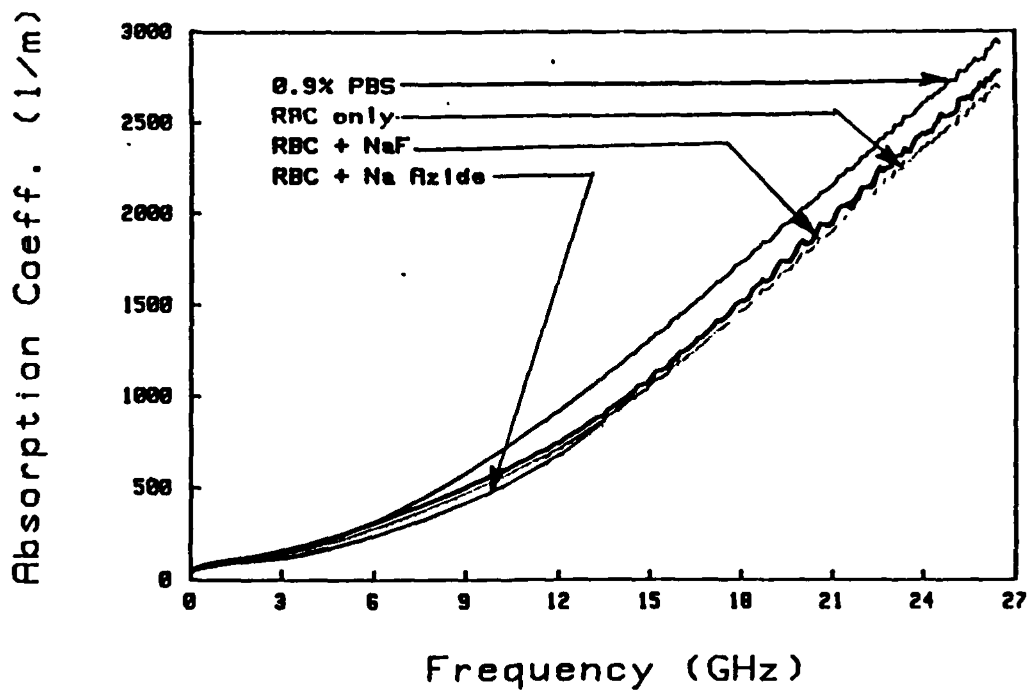
4. Effect of Sodium Azide and Sodium Fluoride on Cell and Hemoglobin Dielectric Constants.

Studies were undertaken to determine effects of metabolic agents on the dielectric properties of human erythrocytes and other cell types. Dielectric spectra of erythrocytes in whole blood, or washed cells, were determined. Cell were then treated with 18mM sodium azide (NaA) or 40mM sodium fluroide (NaF) (ie concentrations known to metabolically inhibit mammalian cells) and dielectric spectra were re-determined over a period of 20h at temperatures in the range 22- to 36.5$^{o}$C. NaA caused significant time-dependent alteration in both $\epsilon'$ and $\epsilon''$, over the entire frequency range, whereas NaF treatment decreased $\epsilon''$ in the frequency range of 50-12000 MHz, but had only a marginal effect on $\epsilon'$. The effect of both agents on the dielectric constants of blood increased with increased temperature. Changes were more pronounced in whole blood than in washed red cells resuspended in PBS. Effects of both agents were evident after 3h of incubation; NaA induced a significantly greater decrease in $\epsilon'$ and $\epsilon''$ than NaF. The effects of 20h incubation; at 36.5$^{o}$C on $\epsilon'$,$\epsilon''$ and $\alpha$ are shown in Figures 6,7 and 8, respectively. NaA caused an approximate 66% reduction in $\epsilon'$ throughout the 100- to 26,400 MHz-frequency range, whereas NaF reduced $\epsilon'$ by only about 2%. NaF treatment increased $\epsilon''$ in the frequency range of 100- to 12,000 MHz, with a maximum increase of 33% at 3000 MHz. NaA treatment, on the other hand, reduced $\epsilon''$ by approximately 27% over the entire frequency range. These trends were also evident in the measurement




Loss Factor (e'') of RBC, RBC + NaF, and RBC + NaAzide which are suspended in 0.9% PBS buffer. T = 36.5 °C.

Figure 7




Absorption Coefficient of RBC, RBC + NaF, and RBC + NaAzide which are suspended in 0.9% PBS buffer. T = 36.5 °C.

Figure 8

of $\alpha$, as shown in Figure 8. A more detailed analysis of these, and replicate data, will involve determination of $\Delta\alpha$, the difference in the absorption coefficient between treated and untreated red cell suspensions and the solvent phase.

Two possible mechanisms for the differential effect of NaA on erythrocyte dielectric properties were considered: a) electrostatic interaction of NaA with intracellular hemoglobin tetramer, and b) cell metabolic alteration resulting in altered cell structure and/or function. We have conducted preliminary investigations of the effect of 18mM NaA on the dielectric properties of human hemoglobin suspensions at 37°C and have been unable to induce the change in $\epsilon'$ or $\epsilon''$ detected upon treatment of erythrocytes with this agent. This leads to the suggestion that the effect was on the cell _per se_, possibly related to alteration in membrane active transport, a known result of NaA treatment. The relatively smaller magnitude effect of NaF, an agent which affects cell energy metabolism via inhibition of glycolysis causing decreased ATP and $K^+$ efflux, suggests the possiblity of event specific effects on cell dielectric properties. There are no previously reported effects of altered cell energy metabolism on dielectric properties in the frequency range 50- 27000 MHz. Such effects are not predicted by Debye dispersion theory. It may be noted that cell energy metabolism is temperature-dependent. Hence, the anomalous effects of variation of temperature on the dielectric properties of erythrocytes, reported above, could possibly also be related to altered energy metabolism.

In an attempt to obtain additional information about the relationship of dielectric properties to cell membrane transport we investigated the effects of ouabain, (0.1 mM) on erythrocytes. Ouabain treatment increased $\epsilon'$ and $\epsilon''$ of cells at 37°C, which provides additional evidence that cell metabolic events, such as membrane cation active transport, may be reflected by changes in cellular dielectric properties.

5) Cell Dielectric Spectra

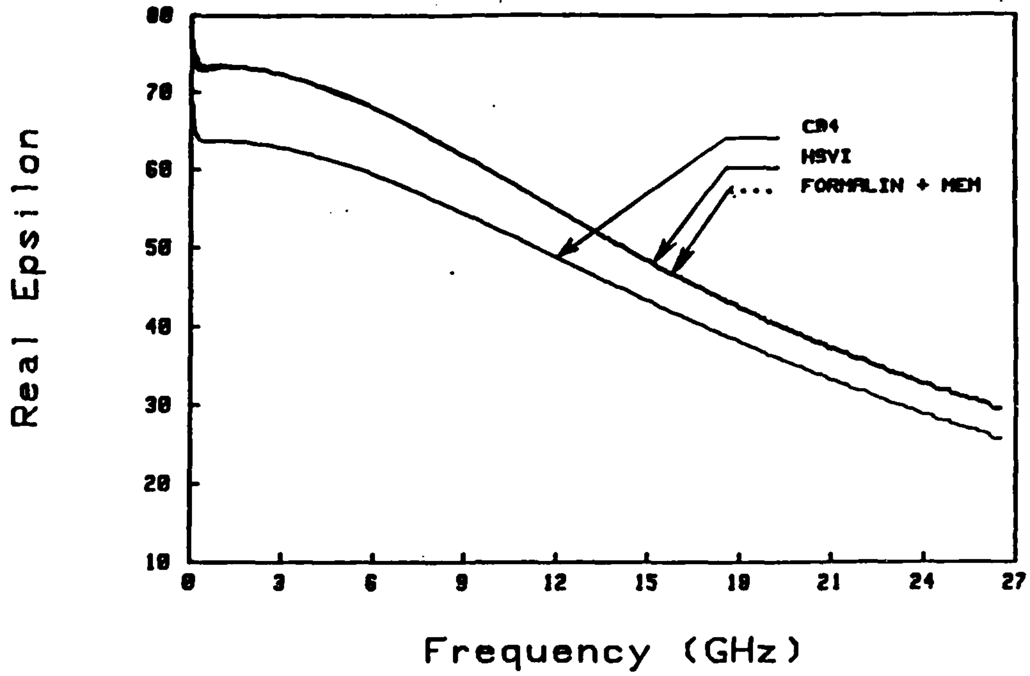
Dielectric parameters ($\epsilon'$,$\epsilon''$ and $\alpha$) of a number of different cell lines were measured over the 50 - 26400 MHz range in an attempt to identify cell - type - specific dielectric properties. Comparison of these spectra will also provide a means of detecting dispersions or absorption bands related to DNA resonance. In addition to human erythrocytes and lymphocytes we have measured the dielectric properties of HeLa, glioma (LN-71, L929), MKTD, RT-2, YAC, VERO, and CHO cells.

6) Dielectric Properties of Viruses

The real and imaginary part of the complex dielectric constant ($\epsilon'$,$\epsilon''$) and the absorption coefficient ($\alpha$) of herpes simplex (HSVI), coxsackie (CB4), and an adenovirus (A7) were determined for the frequency range 50-26400 MHz. The high frequency dispersion of $\epsilon'$ at 25.5°C for suspension medium (ie minimum essential media, MEM), CB4 in MEM ($10^{10}$ virus particles/ml), and HSVI ($2.2 \times 10^7$ virus particles/ml) in MEM are shown in Figure 9. The addition of 1% (v/v) formaldehye induced time-dependent changes in $\epsilon'$ and $\epsilon''$ for CB4 suspension, with minimum effects on HSV1 suspensions. Figure 10 indicates the decrease in $\epsilon'$ for CB4 treated with formalin relative to MEM + formalin and HSVI + formalin, 3h after the addition of formalin to the media. The data suggest that the effect of formalin on viruses may be due to alterations that are dependent upon viral composition and/or structure. CB4 is a 30nm diameter nonenveloped virus with a single-stranded RNA genome having a molecular weight of $2.5\times10^6$ Daltons. HSVI is a 100-200 nm diameter enveloped virus with a double-stranded DNA genome of 80- to 150 x $10^6$ Dalton molecular weight. Differences in the dielectric properties of these virus after treatment with formalin may tentatively be attributed to differences in molecular structure. Treatment of HeLa cells (4.7 x $10^6$ cells/ml) with formalin at the same concentration did not detectably alter the dielectric properties of the cell suspension.

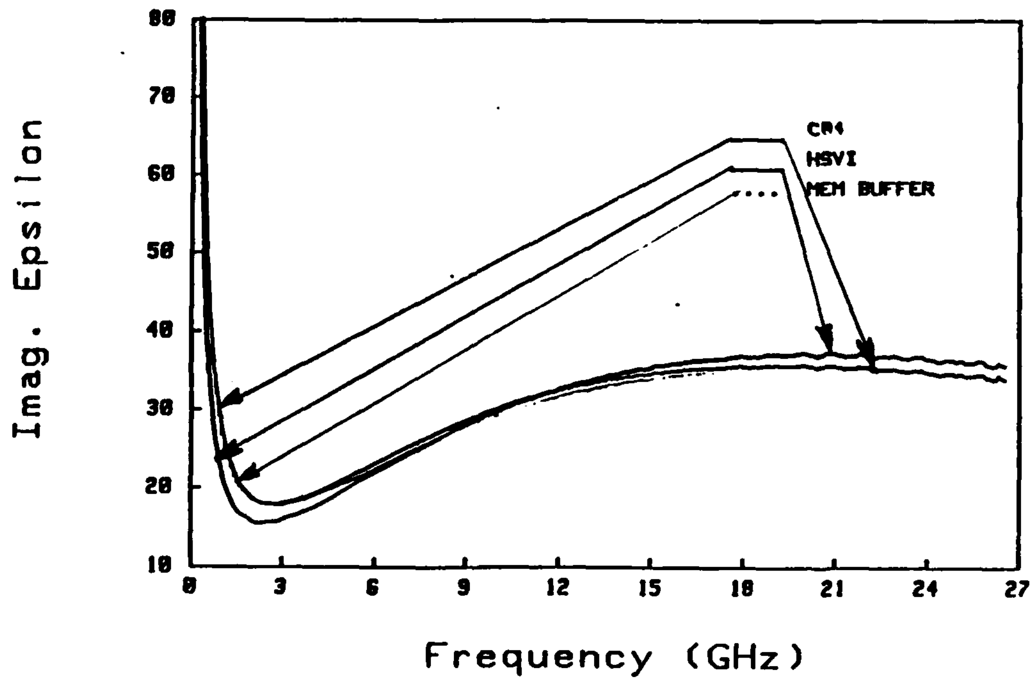
7. Treatment Effects on Cellular Dielectric Dispersions

In addition to the treatment effects discussed above, preliminary studies have been undertaken of the effects of anesthetics (procaine and ketamine) and the calcium ionophore (A23187) on erythrocyte (anesthetic agents) or glioma (A23187) cell suspension dielectric properties. These agents did not induce detectable displacements of the $\epsilon'$ or $\epsilon''$ dispersions. Comparison of the dispersions of erythrocytes treated with procaine and ketamine revealed differences in contours in the 1-6 GHz region. The significance of




Comparison of Permittivity (e′) of CB4 and HSVI Cells fixed in Formalin in MEM buffer. Temperature was 26 °C.

Figure 9




Comparison of Loss Factor (e′′) of CB4 and HSVI Cells in Medium of MEM. Temperature was 25.5 °C.

Figure 10

these differences will be investigated further.

8. Quasi-Steady State Laplace Equation

The quasi-steady state solution of Laplace's equation, which was programmed for digital computation, is being applied to a determination of the dielectric properties of cell monolayers in the 50 - 26400 MHz range. The solution permits calculation of the RF induced potentials in multilayered spheres of arbitrary dielectric constants suspended in lossy dielectric liquid. We will utilize measured values of the complex reflection coefficient ($R^*$) of $60\mu$ dextran spheres (Cytodex beads) with, or without, an adherent layer of CHO cells, to determine $\epsilon', \epsilon''$ and $\alpha$ for the Cytodex spheres and the CHO monolayer cells.

9. Spectral Analysis

A primary objective of this investigation has been to detect resonant or anomalous absorption or dispersions in mammalian cells that may be related to previously reported DNA resonant or enhanced absorption in DNA suspensions in the 1- to 10 GHz-frequency range. Preliminary analyses of dielectric spectra of the various cells and viruses we have investigated to date have not revealed evidence of resonant absorption in this frequency range. Comparisons of the dispersions have, however, revealed small unexplained differences in the spectral shape of $\epsilon'$, and in some instances $\epsilon''$, in the 1- to 6 GHzrange. Due to: a) the relatively small magnitude of these effects, b) the current unavailablity of replicate spectra, and c) possible calibration artifacts due to uncompensated impedance mismatches in the measurement system, we cannot associate these differences with cellular dielectric properties *per se* at this time. A detailed analysis and comparison will be made of the difference in absorption coefficient ($\Delta\alpha = \alpha$ (cell suspension) - $\alpha$ (Medium)), $\Delta\epsilon'$, and $\Delta\epsilon''$, in order to determine if the observed sprectral characteristics are artifactual.

10. Cell Synchronization

The technique of double thymidine block has been used to synchronize CHO cells. The dielectric spectra of synchronized cells in various stages of the cell cycle ($G_0$, $G_1/S$, S, $G_2/M$) have been determined and compared with spectra of asynchronous CHO cultures. Preliminary analysis indicates cell cycle dependent changes in $\epsilon'$, with associated smaller magnitude changes in $\epsilon''$. Additional analyses and replication will be employed to establish the validity of this effect.

## SUMMARY

During the first year of study evidence has been obtained that the dielectric properties of mammalian cells and viruses are dependent upon metabolic status, as well as other factors. On the basis of dielectric theory these observations are unexpected and unexplained. Since, to our knowledge, such studies have not been previously conducted in this frequency range, comparisons are not possible. Further investigation, including replication of data described herein and additional spectral analyses will be conducted during the next contract period. The results of these studies will serve as the basis for the design of experiments to achieve the other objective of this investigation, namely the determination of action spectra for cellular alterations by radiofrequency radiation.

## Publications

Liu, L.M. and Cleary, S.F. (1988): Effects of 2.45 GHz-microwaves and 100 MHz-radiofrequency radiation on liposome permeability at the phase transition temperature. Bioelectromagnetics 9:249-257.

Liu, L.M., Cleary, S.F., Rhee, K., Czerska, E. and Davis, C. (1988): Dielectric spectroscopy of mammalian cells. Abstracts of the 10th Annual Meeting of the Bioelectromagnetics Society, Stamford, Conn. June 1, 1988, p. 51.